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THE USE OF DOUBLE TRANSLOCATION TO CONTROL POPULATIONS OF THE G--ETC(U)
MAY 78 M H ROSS, D G COCHRAN N00014-77-C-0246

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Research objective

The research accomplished during the first of this three year investigation was conducted according to four specific objectives. >The first involved procedures for sexing and identifying double males. Two hundred individuals were selected/hr, by the most satisfactory method. >The size of the crossing systems needed to produce specific numbers of double males was also estimated. Objective 2 dealt with competitiveness studies. We found that unmated males remained competitive during the first mo of adulthood, reaching a slight peak at ca 2 wks. Another series of experiments showed laboratory translocation strain males tended to out-compete males collected from the USS J F Kennedy. Objective 3 involved the choice of the particular double translocation stock for field tests. A new double, showing complete male sterility and high competitiveness, was chosen in preference to an older stock, T(3;7;12), which has lower sterility. Under Objective 4, a population experiment using releases of T(3;7;12) males into the Kennedy strain was initiated. Data, though incomplete, leave little doubt that releases into one generation of cockroaches will result in population suppression in the next. ✕

This year's studies, especially those involving field-collected cockroaches, auger well for the success of field tests. Stock buildup, additional studies of shipboard populations, and arrangements with Navy entomologists for field tests (goals for 1978) should place us in readiness for a field trial in 1979.

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ANNUAL REPORT NO. 1

The Use of Double Translocations to Control
Populations of the German Cockroach

by

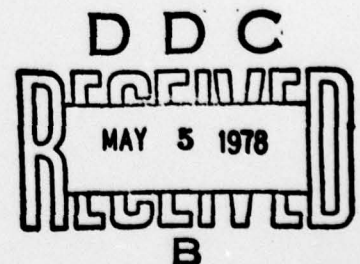
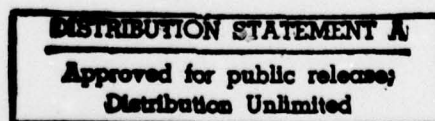
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1 May 1978

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In the course of earlier investigations, a unique type of sterility mechanism, "embryonic trapping", was discovered in the German cockroach, *Blattella germanica* (L.) (Ross and Cochran 1976). Matings of males carrying a high genetic load, such as that due to heterozygosity for two translocations, can reduce the number of viable embryos in an egg case to a point at which their combined strength is insufficient to force it open at the time of hatch. The first type of double male to be developed showed full competitiveness and caused sterility in 70% of the oothecae (Ross and Cochran 1977). Sequential releases suppressed growth of a laboratory wild-type population (Ross 1977). The third double to be developed, T(3;7;12), also showed good competitiveness along with ca. 90% sterility. In addition, it appeared we had the capability of producing males with at least 98-99%, if not complete sterility, by combining T(8;9) with T(4;8;10). Such mechanisms could be useful in an integrated program for cockroach control. The rationale behind this approach is that relatively small releases could be used to prevent the re-development of large infestations following treatment of ships or other areas. The overall goal of the present Contract is to test this hypothesis.

This investigation was planned on a 3-year basis, with 2 years of laboratory research and development to prepare for field tests in 1979. The work accomplished during the first year (3/1/77-2/28/78) is summarized here according to the objectives stated in the original proposal. However, Objective 2 is expanded to include competitiveness studies of field-collected vs laboratory males (Obj. 2B). The description of results as detailed under the 4 objectives is followed by a discussion of their significance in respect to field trials. The population experiment initiated under Obj. 4b will be completed during the 2nd year of this Contract (3/1/78-2/28/79).

Objective 1 - Sexing and identifying double translocation heterozygotes

The translocations used in this study do not have phenotypic effects. Mutants showing close to complete linkage are used for identification. The single stocks that are crossed to produce the doubles are maintained by backcrosses to an appropriate marker. In these systems, backcross progeny showing a normal phenotype are the translocation heterozygotes. Likewise, the double males among intercross mutant phenotypes, one for each of the parental translocations. For example, progeny showing black-body and/or rose-eye are discarded in the process of selecting T(8;9), T(4;8;10) double males. Those with orange-body and/or hooded pronotum are discarded in the case of the T(3;7;12) double.

We experimented with three selection procedures, as follows:

(a) Nymphs were sexed and phenotyped in one operation, using low power of a binocular microscope. Groups of 30-50, usually 4th instar, were anesthetized briefly with CO₂, scooped up on a piece of paper, and quickly scanned for normal-colored males. Those with normal eye color

in the case of T(8;9), T(4;8;10) or normal pronota in T(3;7;12) were lifted gently with a fine-pointed forceps by a leg or antenna and dropped in a clean jar. The remainder were discarded. The process was repeated until all double males had been separated from the particular set of backcross progeny. From 300-350 T(8;9), T(4;8;10) males were separated accurately and without injury within 1 hour. T(3;7;12) selection is slower, due to the more careful check required for identification of hooded-pronotum. However, 150-200 can usually be done within the hour.

(b) Backcross progeny were allowed to reach the terminal (6th) instar. They were then emptied from their container into a 2.8 mm mesh sieve (No. 7) and agitated gently for several minutes. The smaller nymphs (largely, but not entirely males) descended into the lower pan (Dicken's method). They were then anesthetized and phenotyped as above, also checking for sex. This method was considerably slower than "a" due to the sieving process.

(c) Large male nymphs were separated using a manual aspirator. They were identified by the long, slender shape of the abdomen. Phenotyping for T(3;7;12) was as in "a". In T(8;9), T(4;8;10), black-body hybrids could be removed by the aspirator, as the body color is easily distinguished from the "normal" color of the double males. This method was also slower than "a" (the nymphs do not remain quiescent when approached with the tube of an aspirator).

We have found "a" to be the most efficient method of separating double males for release. It has the additional advantage that the males can be separated when about half-grown, or, if desirable for release purposes, at a somewhat earlier stage.

Objective 2 - Studies of male competitiveness

2a. The effects of age on competitiveness of unmated males

The competitiveness of unmated males ranging in age from 5 to 34 days post maturation was tested against males which had recently reached sexual maturity (3-4 day-old males). In one set of experiments, the older males were wild type (+/+); in the other, T(4;8;10) were the older. The results are summarized in Table 1. The males remained competitive throughout the first month of adulthood, although a very slight drop may have occurred after 20-25 days. The normal males reached peak competitiveness at 14-16 days; T(4;8;10) apparently peaked somewhat earlier, although the very high degree of success at 11-13 days is out-of-line with the other data. Additional tests were made placing 11-13 day-old T(4;8;10) in T/+; 12 were by +/+ males ($\chi^2 = 2.0$, $P > 0.20$). Although it does appear T(4;8;10) increases in competitiveness somewhat ahead of +/+, we believe the results of the first tests were spuriously high.

The above findings raised an important question, namely, would previously mated males within a population also show the high degree of competitiveness exhibited by the unmated males? The study was

Table 1. Competitiveness of unmated males during the first month of their adult life span.

Age of older male ^a (da)	No. tested:		% matings by:		χ^2 for equal competitiveness	
	+/+ older	T(4;8;10) older	+/+ older	T(4;8;10) older	+/+ older	T(4;8;10) older
5-7	-	12	-	50		0.00
8-10	13	19	46	53	0.08, P > .70	0.05, P > .80
11-13	11	15	55	87 ^b	0.09, P > .70	8.07, P > .01
14-16	15	14	67	64	1.17, P > .20	1.14, P > .20
17-19	42	26	55	54	0.38, P > .50	0.15, P > .50
20-22	32	13	53	58	0.12, P > .70	0.69, P > .30
23-25	15	16	53	50	0.07, P > .70	0.00
26-28	10	22	40	59	0.40, P > .50	0.72, P > .30
29-31	13	23	46	39	0.08, P > .70	1.09, P > .20
32-34	14	20	50	50	0.00	0.00

^aTested against males aged 3-4 days post maturation.

^bResults spuriously high. See text for additional data.

expanded to at least acquire some data bearing on this problem. Normal males were caged with $+/+$ virgin females for 10 days following maturation. They were removed on day 11 and each placed with a 3-4 day-old T(4;8;10) male and a virgin $+/+$ female. Of 28 matings, 21 were T/+ and 7 were $+/+$, $\chi^2 = 7.0$, $P < 0.01$. In contrast, unmated 11 day-old $+/+$ males showed 55% success (Table 1). The mated males were relatively young and had mated only once. It seems likely that still older mated males would be even less aggressive. Nevertheless, in planning numbers for release in field tests, we will assume a limited amount of competition from the older adult males as well as newly matured, unmated males (also see Obj. 4b).

A second finding with significance to field trials was that males obtained maximum competitiveness at ca. 2 wks of age or, in the case of T(4;8;10), a few days earlier. Originally we planned to release male nymphs which would mature more or less synchronously with oncoming nymphal groups in the resident population. It now appears an advantage may be derived from releasing adult unmated males as well as nymphs.

2b. Competitiveness of field strain vs laboratory-reared males

A collection of live cockroaches from the USS J F Kennedy was obtained in November 1977, through the cooperation of LCDR Jay Lambdin. It included 69 large nymphs. These were used for competitiveness studies; the remainder were used for a population experiment (Obj. 4b).

The large nymphs were sexed and allowed to mature. T(4;8;10) male nymphs of similar size were selected from their backcross system. At 4-5 da post-maturation, each field strain male was caged with a T(4;8;10) male of matching age. A field strain female, aged 4-6 da post-maturation, was added. One mo after the experiment was initiated, the ootheca was examined to determine the mating type. The results of 30 observations are as follows: 10 showed normal embryonic development (field strain male successful); 20 showed lethality indicative of a translocation (mating by T(4;8;10) male). These data indicate significantly higher competitiveness of the laboratory strain at the 10% level of probability, and are close to significance at the 5% level ($\chi^2 = 3.34$, compared to 3.8 for 5% significance).

Objective 3 - Synthesis and study of T(8;9;10)7i and T(8;9),T(4;8;10)

These two doubles were the only two which could be developed within a single year that we felt might equal or surpass the usefulness of T(3;7;12) for control purposes. T(8;9;10)7i is a similar type of double to T(3;7;12) and, other properties being equal, it would have an advantage in respect to handling. Its identification involves use of the B1 and ro markers which, as already noted, make possible speedier selections than in a system involving hd, i.e., T(3;7;12). The other new double, T(8;9),T(4;8;10), also uses these markers. Neither double could be developed until we had synthesized a stock of T(8;9) marked with B1 (chromosome 8) and homozygous for ro (chromosome 10 marker used to identify both T(9;10)7i and T(4;8;10). Once this was accomplished, precedence

was given to development of T(8;9),T(4;8;10) for the following reasons: (1) preliminary chromosome analyses indicated the males should show complete sterility, whereas it seemed unlikely the lethality in T(8;9;10)7i would be that high, (2) this double includes the translocation, T(4;8;10), of which the males tended to out-compete field-collected males (Obj. 2b), and (3) the T(4;8;10) parental stock was already very large, as compared to a comparatively small stock of T(9;10)7i.

3a. Synthesis

The new T(8;9) stock was developed by standard backcrosses to a double mutant stock of rose-eye and black-body (ro/ro,Bl/Bl), as outlined on p. 26 of the original proposal. Among the backcross progeny, those showing the phenotype of Bl hybrids were T(8;9). Those with rose-eyes were selected and used to initiate and expand a continuing series of backcrosses to Bl/Bl,ro/ro. It required ca. 9 mo to build a reasonably strong backcross system. At this point, we began to use all T(8;9) males for crosses to T(4;8;10) females, with continuation of the backcross system depending on T(8;9) females only. Intercrosses to T(9;10)7i females were begun about 2 mo later.

An average of one double male was produced for every 2 females from first egg cases in the T(8;9) X T(4;8;10) intercrossoes. In 2nd egg cases, the mean was closer to 1 double male/3 females. Scoring of 700 intercross progeny showed a 1:1:1:1 ratio between the 4 phenotypes, i.e., normal (double Tls); Bl hybrid (T(4;8;10)); ro (T(8;9)); and Bl hybrid, ro (no Tl). There were no deviations from the expected 1:1 sex ratio. We concluded that no viability loss is associated with T(8;9), T(4;8;10) males.

By the time the first T(8;9;10)7i doubles were developed, the competitiveness tests of T(8;9),T(4;8;10) males were giving such excellent results (3b) that there seemed little purpose to pursuing the study of T(8;9;10)7i. Limited data were obtained, as noted below, but we cannot say whether T(8;9;10)7i would be an adequate substitute for T(3;7;12) - a point of little immediate interest since we plan to use T(8;9),T(4;8;10). Scoring of 80 intercross progeny suggested a deficiency of double males, but the data were not statistically significant.

3b. Analysis of T(8;9;10)7i and T(8;9),T(4;8;10)

As noted above, the work under this section was concerned mainly with the T(8;9),T(4;8;10) double.

Male competitiveness. T(8;9),T(4;8;10) males were tested against laboratory wild-type males by procedures described under Obj. 2b. Among 56 matings, 22 were normal and 34 were by the translocation-carrying males. The results verge on significance at 5%, with a χ^2 well below that expected at 10% ($\chi^2 = 3.3$, $P < 0.10$). These data are compared to similar studies of other doubles as follows:

Stock	Matings:		% success of double male
	w.t.	T,T	
T(8;9),T(7;12)*	31	26	46
T(8;9),T(3;12)	27	28	51
T(3;7;12)	23	27	54
T(8;9),T(4;8;10)	22	34	61

*Previously studied doubles not selected for possible field testing due to comparatively low sterilities, i.e., 70% as compared to 90-100% in those studied under this Contract.

These results, as well as competition studies of T(4;8;10) vs field-collected males (Obj. 2b), lead us to believe T(8;9),T(4;8;10) males are more competitive than field strain males.

Hatch, lethality and sterility. Females which had mated with T(8;9),T(4;8;10) males in the course of the competitiveness tests were saved. Embryonic trapping resulted in complete sterility. The lethality responsible for this effect was estimated as the average no. of embryos that died before completing development divided by the total no. of embryos/egg case. Fully developed embryos that died due to trapping were easily distinguished from those that died earlier by coloration, segmentation, appendage development and eye development. The mean lethality in matings of the double males was $81.0 \pm 1.0\%$. This agrees well with chromosome analyses (see below) and the occurrence of complete sterility (Keil and Ross 1978).

Ten matings of T(8;9;10)7i to wild-type females proved to be completely sterile. However, since chromosome analyses showed a lethality similar to that of T(3;7;12), it is probable a larger series of crosses would have shown low level hatch to occur.

Chromosome analyses. As we have noted previously, two separate translocation configurations appear in cells of the T(8;9),T(4;8;10) double. Chromosome 8 was mis-identified in T(4;8;10), but we have continued to use this designation pending definitive identification of the unknown chromosome. Chromosome segregation at metaphase I in the T(8;9) configuration showed 60% alternate (viable) and 40% adjacent (lethal) types. That in T(4;8;10) showed 32% alternate and 68% adjacent, giving an overall lethality of 80.2% in the double males. This agrees reasonably well with embryonic lethality (see above).

Disjunction in the ring-of-six chromosomes found in meiotic cells of the T(8;9;10)7i double males showed 76% adjacent and 24% alternate types. This should approach, but not reach the complete sterility found at 80-81% lethality (Keil and Ross, 1978).

Objective 4 - Stock buildup and laboratory population studies

4a. Stock buildup

The procedure outlined below was developed to expand the parental stocks, T(3;12) and T(7;12), used to produce T(3;7;12) males for the population study (4b). A similar method is being used to expand T(4;8;10) and T(8;9) to prepare for synthesis of the males needed for field tests.

- 1) Backcrosses for maintenance of T(7;12) and T(3;12) were set up in glass battery jars (capacity 3.8 l, i.e. 1 gal.).
- 2) The date at which adult females first appeared was noted on each jar.
- 3) Oothecae of the females were checked for semisterility at 1 mo post-maturation to insure all progeny were from T/+ matings.
- 4) Females from T/+ matings were placed in a clean battery jar, usually ca. 250-300/jar.
- 5) Newly hatched nymphs were collected weekly from jars in which hatch was occurring.
- 6) Females retaining egg cases after nymphs were removed (#5) were kept for nymphal collection of the following week; those that had dropped their first ootheca were separated and kept for hatch of 2nd egg cases (ca. 1 mo after hatch of 1st egg case).
- 7) At 4-5 wks of age, T/+ nymphs were selected by phenotype (normal pronota in the case of T(3;12) and normal body color for T(7;12), and used for a new set of backcrosses (#1).

Backcross selections (#1, 5) are the most time-consuming step. Nevertheless, two technicians, one sexing and the other phenotyping, routinely set-up backcrosses of the T(4;8;10) translocation that total 1800-2000 nymphs in 3 1/2 hrs. T(7;12) selections, involving or, are equally rapid. T(3;12) is slower, but we have not timed it precisely since we are concerned primarily with T(8;9;10) and T(8;9) stocks, i.e., parental stocks of the double we plan to use in field tests. T(7;12) and T(3;12) backcross systems were cut back as soon as they were not needed to produce T(3;7;12) males for a population experiment (4b).

4b. Laboratory population experiments

The original plan was modified in that T(3;7;12) males were released into a population developed from a freshly-collected field strain rather than a laboratory wild-type strain.

Two collections from the USS J F Kennedy were forwarded by LCDR Lambdin. One was preserved; the other live. The former had nymphs in most instars (71 5th & 6th I; 73 3rd & 4th I; 32 1st & 2nd I). The live

collection was disappointing in that the only nymphs other than 69 late instar nymphs were 16 very small nymphs. We decided to use the large nymphs for competitiveness studies (Obj. 2b) as LCDR Lambdin thought he could obtain another collection with a nymphal distribution more suitable to a population experiment. Unfortunately, most of the cockroaches from the 3rd try (USS John King) died before arrival. The Kennedy strain adult females had, in the meantime, given rise to a very synchronous group of 355 nymphs. We decided to use these for the experiment, although the large number in the single age group forced us to undertake a larger intercross system for producing double males than we had anticipated.

A large glass container, ca. 80 l capacity, was used for the population experiment. The bottom of the jar was fitted with several feet of closely crimped wire screen, ca. 6 cm in height. A 2nd layer of loosely folded squares of wire screen, ca. 8 cm in height, interspersed with wads of paper towels, provided additional harborages. A petri dish with a wet sponge and paper cups of dog food supplied water and food.

The study was initiated by placing the 20 field-collected adult females, 16 field-collected nymphs, and 355 nymphs hatching from the females in the large container (Table 2, 12/15). These were given 2 wks to adjust to their new situation before the first releases were made (1/3). The target nymphal group here was that of 16, which had by then diminished to 13. Releases aimed at the large nymphal group (355) were made on 1/9 and 1/16. An 83% mortality during nymphal development was assumed on the basis of previous study, but mortality between hatch and formation of 1st egg cases by the females was subsequently estimated at 59% (see below). The old adult females were removed (1/3) to determine numbers from their 2nd nymphal group (not necessarily 2nd oothecae as the females may have produced a 1st egg case before collection). The new nymphal group of 211 was returned to the container. Releases to meet this nymphal group were made on 2/13 and 3/2 (Table 2). The releases consisted of males of mixed age, ranging from 5th-6th instar to 2-wk old adults, all equal to or older than nymphs of the target group.

The effects of the releases were assessed by removing females with oothecae every 3 wks and examining them when mature to determine mating type and, subsequently, sterility vs hatch. The results to date are summarized in Table 3. For convenience, the three target nymphal groups are designated as A, B, and C, as listed in Table 3. Six females of group A formed egg cases, of which 4 were double-translocation (T,T) and sterile. Two were indeterminate as the females died soon after removal from the population. In group B, 104 oothecae were recorded, of which only 1 was a wild-type (+) mating, but 3 had lower lethality than expected for the double translocation. The results from group C are as yet incomplete, but it can be predicted with reasonable certainty that this group will be unable to replace itself, similarly to the results for group B. Maximum hatch from all oothecae of group B females is estimated at less than 180---a significant suppression since the parental group on hatch numbered 355. One advantage of a 3-chromosome double, such as T(3;7;12), is that all progeny are heterozygous for one or the other parental translocation. Thus high sterility would persist for at least another generation, maintaining suppression for ca. 7-9 months, and possibly longer.

Table 2. Summary of population growth and releases.

Date	Composition of population	Releases: no. and approx. ratio	
12/15	20 Ad ♀; 16 3rd-4th I; 355 1st I		
1/3	; 13 5th-6th I; 355 3rd-4th I (Old ♀ removed for hatch 2nd ootheca)	70 ♂	10:1
1/9	13 5th-Ad; 295 5th I; 211 1st I	510 ♂	
1/16	13 Ad; 295 5th-6th I; 211 1st-2nd I	259	
		769	6-7:1
1/25	13 Ad; 200 6th-Ad; 175 2nd-3rd I		
2/13	(removed 6 ♀ oothecae from grp of 13)		
	200 Ad; 175 4th-5th I	540	
3/2	200 Ad; 175 5th-6th I, few Ad	311	
		851	9:1

Table 3. Results of egg case observations.

Nymphal group	No. and type of egg case*	% sterile egg cases	No. nymphs hatched
A (16)	4 T,T	100	0
B (355)	100 T,T	93	25
	3 ?T,T*	0	21
	1 +	0	29
			75 Total
C (211)	Data incomplete		

*Translocation-type egg case but lower lethality than expected for mating of double male.

It may be helpful to view these results from the perspective of estimates of population growth and numbers if no releases had been made. Total numbers in the experimental population are estimated on the basis of observed sterility effects; those in an unchecked population from wild-type population data. The first series of releases (1/9-1/16) increased the total number in the population by a factor of 1.6X. Due to these and later releases, the total number remained larger than that of the original population through March, although numbers were smaller than expected if the population had not been checked by sterility effects. During April and May, the estimated hatch in the latter would be ca. 8,000, compared to less than 200 in the experimental population. During these months, the released males are expected to die from old age, with rapid decrease in numbers to less than in the original population. The unchecked population is estimated at ca. 21X that of the original population of 391. Population suppression will have been achieved by releases into only one generation of progeny.

Discussion and Conclusions

A major step was made toward conducting a well-planned field trial through the work carried out under the first year of this contract. Also, the results obtained from competitiveness and population experiments auger well for the success of such attempts. The most significant findings are as follows:

1. Unmated adult males remain fully competitive for 1 mo post-maturation, reaching peak competitiveness at ca. 2 wks. In contrast, mated males apparently suffer a loss of competitiveness.
2. Laboratory strain males carrying a single translocation, T(4;8;10), tended to out-compete field strain males in laboratory tests.
3. Release of T(3;7;12) males into a field strain population suppressed population growth. Sterility and death of released males reduced population numbers to less than the original population in the 3rd-4th mo following the initial releases.
4. A new type of double male, T(8;9),T(4;8;10), is characterized by high competitiveness and complete sterility from embryonic trapping.
5. Measurements of hatch in parental translocation systems and inter-crosses can be used to plan the timing and extent of rearing procedures needed for production of specific numbers of sterile double males.

In earlier laboratory population experiments, released males competed successfully with those of the population when they were matched rather precisely with respect to age (Ross 1975, 1976, 1977). This would be difficult in the field. The studies reported here indicate that older, unmated males can compete equally with those maturing within a population. Double males selected from weekly nymph jars could be used for a monthly release containing a mixture of 1-2 wk-old adults and 5th-6th instar nymphs, as in the population study (Obj. 4). Thus, mating failure that

might occur if the wild-type males matured ahead of released males can be avoided. Also, it will not be necessary to have all released males of the same age, thus reducing the required crossing systems.

The most widely recognized reason for lack of success in field tests of sterile males or other genetic mechanisms is the failure of released males to mate with females of a target population. Several factors may be involved. The process of sterilization by radiation or chemosterilants may reduce fitness. This is avoided by using embryonic trapping to achieve sterility. Laboratory colonization can also result in a loss of competitiveness. In addition, genetical and/or behavioral differences affecting mating may originate through differences in geographical origin of the laboratory vs field population. The studies reported here indicate these problems are unlikely to affect field tests of the double-translocation carrying males. Indeed, there is reason to believe the double males chosen for field testing, i.e., T(8;9),T(4;8;10), are capable of out-competing field strain males.

At the start of this Contract, data were already on hand that indicated T(3;7;12) was a highly promising genetic mechanism. However, it had not been tested in a population experiment. We felt it would be more valuable to determine whether releases would suppress growth of a population from a freshly-collected field strain than that of a laboratory wild-type population. The suppression already evident following releases of T(3;7;12) males into the population started from the Norfolk collection (Kennedy) is very encouraging.

The choice of T(8;9),T(4;8;10) over T(3;7;12) was a judgment decision. Competitiveness is of primary importance, and the comparison of competitiveness tests indicated the T(8;9),T(4;8;10) males showed the better performance in tests with laboratory wild-type females. Also, this double stock includes the translocation, T(4;8;10), of which the males tended to out-compete field-collected males. Other reasons for selecting this double were (a) crossing and selection procedures can be accomplished more rapidly, (b) double males can be selected in very small nymphs (hd for T(3;7;12) is hard to identify prior to the 4th instar), and (3) sterility is complete. The primary advantage of T(3;7;12) is that it requires about 1/2 as many intercrosses to produce a given number of double males, but we feel this is outweighed by other factors as noted above. The present stock of T(4;8;10) is already sufficiently large for setting up intercrosses to produce close to 200 double males/week, providing all T(4;8;10) females were used for crosses to T(8;9) males.

In summary, we have developed highly promising genetic mechanisms in the two double translocations considered for possible field tests, shown that males of such stocks compete equally and possibly out-compete field strain males, and demonstrated they are capable of suppressing growth of a freshly-collected field strain population. Prior to field testing, we need to learn more concerning natural populations, to build up the genetic crossing systems, to obtain a rough estimate of numbers needed for release, and to convince those directly involved with the releases that they are serving a useful purpose. These are goals for 1978.

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